

Effect of Gonadotrophin (Diclair®) on Semen Characteristics, Body Conformation and Hormonal Profile of Mature Male Turkeys

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Abstract— Sixteen sexually matured (12 months old) healthy male turkeys were used to determine the effect of Gonadotrophin (Diclair®) on semen characteristics, body conformation and hormonal profile. The turkeys were divided into 4 treatment groups of 4 turkeys per group, identified as T_1 (control), and ministered with 1.00ml physiological saline, T_2 , administered with 13.50i.u Diclair®, T_3 , administered with 27.00 i.u Diclair® and T_4 , administered with 40.50i.u Diclair®, with one turkey per replicate in a completely Randomized Design (CRD). The injections were divided into three doses each and administered intramuscularly in the thigh for three consecutive days. Semen was collected one week after Diclair® administration, twice a week for 4 weeks by the abdominal massage and manipulation of the cloaca method. Four cocks were randomly selected from each treatment group and bled one week after Diclair® injections to collect blood for hormonal profile evaluation. 30 days after Diclair® injection, parameters for body confirmation were measured. The results showed that there were significant differences ($P < 0.05$) among the treatment groups in all the parameters for semen characteristics except semen pH and semen volume which were similar ($P > 0.05$) among the treatment groups. The results further showed that there were significant differences ($P < 0.05$) among the treatment groups in all the parameters for body confirmation: wing length, neck length, shank length, body length, beak length, thigh length, keel length, chest circumference and tail length. Similarly, the results showed that there were significant differences ($P < 0.05$) among the treatment groups in follicle stimulating hormones (FSH), luteinizing hormone (LH) and testosterone levels. The results of this study suggest that Diclair® improved semen quality, body confirmation and was not detrimental to the hormonal profile of the turkeys.

Keywords— Diclair®, semen quality, body conformation, hormones, Turkeys.

I. INTRODUCTION

Turkeys (*Meleagris gallopavo*) are birds that originated in north America, that were domesticated in Europe and are now an important source of food in many parts of the world (Brant, 1998). Turkey occupies an important position next to chicken, duck, guinea fowl and quail in contributing to the most evolving sector, which is playing a significant role in augmenting the economic and nutritional status of varied population (Katie and Frazer, 1988). All over the world turkeys are reared for their tasty and high quality meat (Probakaran, 2003). Hence they are kept because of the economic service they render (Okeudo, 2005) such as eggs, meat, feathers and sometimes pet.

To get the fullest benefits from the breeding turkeys therefore, a good knowledge of their sperm production is essential as well as their sperm output. Sperm producing potentials are evaluated by aspect of semen output: volume, motility of sperm cells, morphology of spermatozoa, proportion of live sperm cells and concentration in ejaculate. No single parameter has been proved to be an accurate predictor of the quality of individual ejaculates (Iheukwumere *et al.*, 2001). Sperm formation involves the use of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Iheukwumere *et al.*, 2004). Most of these preparations of FSH and LH are very expensive perhaps because of the brand names, some of them require cold chain storage and often deteriorate because of inadequate storage and handling (Herbert *et al.*, 2000).

Diclair®, also known as Humeon or Mentrophin and with similar constituents as plusset® is a gonadotrophin preparation lyophilized in vials containing a mixture of follicle stimulating hormone and luteinizing hormone in a ratio 1:1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH in Diclair® play vital role in the initiation of spermatogenesis. The hormone preparation is cheap, readily available and does not require cold chain storage (Iheukwumere, 2005).

It has not been determined if the administration of the hormone preparation for spermatogenesis and semen production would induce any side effects on the body conformation and hormonal profile of the turkeys. This study was therefore carried out to determine the effect of Diclair® administration on the semen quality, body conformation and hormonal profile of mature male turkeys.

II. MATERIALS AND METHODS

2.1 Experimental Birds and their Management:

Sixteen healthy sexually matured male turkeys aged 12 months were used for this study. The turkeys were purchased from the local markets and housed in clean pens. Routine management practices were carried out which include deworming, daily observation of birds to identify sick ones, maintaining clean and dry litter and vaccination against diseases. The turkeys were fed Grower Mash. Feed and water were provided *ad libitum* throughout the 28 days duration of the experiment. They were weighed every week and their weights were recorded.

2.2 Experimental Design and Drug Administration

Sixteen male turkeys were divided into 4 treatment groups consisting of 4 turkeys per group with one turkey per replicate in a Completely Randomized Design (CRD). These groups were assigned to 4 levels of Diclaire® injection as treatments. The levels of Diclaire® were 0.00i.u, 20.25i.u, 40.50i.u, and 60.75i.u Diclaire® represented as T₁, T₂, T₃, and T₄ respectively. The group which received 0.00i.u Diclaire® (T₁) served as the control.

Diclaire® was supplied in 3 vials, each containing FSH 75i.u and LH 75i.u. The content of each vial was dissolved in 1ml of physiological saline solution immediately prior to use, resulting in a solution of DFSH 75i.u plus DLH 75i.u per ml.

TABLE 1
DOSES OF DICLAIR® ADMINISTERED TO MATURE MALE TURKEYS

Day	Treatment Dosage (ml)			
	T ₁	T ₂	T ₃	T ₄
1	0.00	0.03	0.06	0.09
2	0.00	0.03	0.06	0.09
3	0.00	0.03	0.06	0.06
Total	0.00	0.09	0.18	0.27

TABLE 2
CONCENTRATION OF DICLAR® ON MATURE MALE TURKEYS

Day	Concentration of Diclaire® (i.u)			
	T ₁	T ₂	T ₃	T ₄
1	0.00	4.50	9.00	13.50
2	0.00	4.50	9.00	13.50
3	0.00	4.50	9.00	13.50
Total	0.00	013.50	27.00	40.50

All treatments were administered, intramuscularly on the breast muscle of each turkey using a 1ml syringe with 0.01ml graduation.

2.3 Semen Collection and Evaluation

The turkeys were trained to ejaculate by abdominal massage and manipulation of the cloaca as described by Abu *et al.* (2006). By this method, ejaculates were collected from each cock after one week of Diclaire® injections and continued for 4 weeks. This involved a gentle messaging of the abdomen while the opposite hand simultaneously stroked the lower back and tail feathers of a restrained turkey. When phallic tumescence was achieved, the collector's hands were placed around the cloaca with a downward pressure while the lower hand exerted slight upward pressure. The semen which pools on the phallus after each squeeze of the cloaca was collected into a clean dry test tube. To minimize the spread of pathogen care was taken by the collector not to touch the cloacal structure.

Semen evaluation was done as promptly as possible post collection as described by Rodriguez-Martinez and Barth (2007) for qualitative and quantitative parameters such as semen volume, sperm concentration, sperm motility, semen pH, dead sperm percentage and live sperm percentage.

2.4 Body weight and body size measurement

The body weights of the turkeys were measured in kilogram every week using a 20 kg weighing scale and their weights recorded. The body conformation was measured in centimeter using a measuring tape 30 days after Diclair® injection. Wing length was measured from the shoulder joint to the extremity of terminal phalanx, while neck length was considered as the distance between the occipital condyle and the cephalic borders of the ceraerids. Shank length (SL) was measured from the hock joint to the tarsometatarsus digit-3 joints, thigh length (TL) was taken as the distance between the hock joint and the pelvic joint. Body length (BL) was measured as the length of the body from the base of the neck to the base of the tail around the uropigail gland. Back length was measured as the distance between the rectal apterium to the end of the maxillary nail. Head length was taken from the end of the neck to start of the beak. Keel length (KL) was measured as the length of the cartilaginous keel bone or metasternum, and chest circumference was measured under wing at the edge of the sternum. To ensure accuracy, each measurement was performed twice and the mean was used in subsequent analysis.

2.5 Hormonal Assay

Blood samples (5ml each) were obtained with needle and syringe by wing vein puncture of the sixteen turkeys on day 7 after the Diclair® injection, for testosterone FSH and LH evaluation. It was cooled immediately in iced water and transferred to the laboratory, refrigerated at 4°C for 1 hour and the serum separated by centrifugation at 5,000rpm for 10 minutes. The serum was stored immediately at 20°C until enzyme immune assayed (EIA) for testosterone, FSH and LH as described by Micallef *et al.* (1995).

2.6 Data Analysis

Data obtained on semen characteristics, body conformation and hormonal profile of the mature male turkeys were subjected to one-way analysis of variance (ANOVA) using the technique of Steel and Torrie (1980). Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi (1990).

III. RESULTS AND DISCUSSION

The results of Diclair® administration on semen characteristics of mature male turkeys are shown in Table 3. There were significant differences ($P < 0.05$) among the treatment groups in sperm concentration, individual motility, percentage live sperm cells, and percentage dead sperm cells. However, there were no significant differences ($P > 0.05$) among the treatment groups in semen volume and semen pH. The colour of semen collected from individual turkeys was milky-white.

Turkeys on T₁ and T₂ recorded the highest numerical value of 0.33ml in semen volume. The lowest numerical value in semen volume was observed in turkeys on T₂ (0.30ml). The values of semen volume obtained in this study were higher than the range of 0.21±0.1–0.26±0.3ml reported by Abu *et al.* (2006) in cocks, but within the range of 0.25±0.2–0.31±0.14 reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. Semen volume varies with species, age, breed, season and frequency of ejaculation (Ozkan *et al.*, 1998).

Turkeys on T₂ recorded the highest numerical value of 7.10 in semen pH. The lowest numerical value in pH was observed in turkeys on T₁ (7.00). The pH values obtained in this study were within the normal range of 7 – 8 reported by Meacham (2002). The measured pH can depend on the length of time since ejaculation and it tends to increase shortly after ejaculation as a result of loss of CO₂ (Meacham, 2002).

Turkeys on T₄ recorded the highest value of 1.96 ($\times 10^6$ /ml) in sperm concentration and this differed significantly ($P < 0.05$) from rams on T₁ and T₂ which were similar ($P > 0.05$) to each other in sperm concentration. There was no significant difference ($P > 0.05$) between turkeys on T₄ and T₃ in sperm concentration values. The lowest value of 1.72 ($\times 10^6$ /ml) in concentration was observed in turkeys on T₂. The sperm concentration values obtained in this study compares favourably with the reports of Abu *et al.* (2006) who recorded 1.98 – 2.10 $\times 10^6$ /ml; Ezekwe *et al.* (2003) who recorded 1.25 – 2.13 $\times 10^6$ /ml and Oguike *et al.* (2000) who reported 1.18 – 2.13 $\times 10^6$ /ml in Nigerian local cocks, but lower than the value of 3.8 $\times 10^6$ /ml reported by Chalov (1970) in cocks. This variation in sperm concentration of the individual cocks and male turkeys, could be attributed to factors such as breed, (Oguike *et al.*, 2000), plane of nutrition, ambient temperature, frequency of semen collection and drug administration (Abu *et al.*, 2006).

TABLE 3
EFFECT OF DICLAI[®] ON SEMEN CHARACTERISTICS OF MATURE MALE TURKEYS

Parameters	Treatment (Diclair [®] i.u)				
	T ₁ 0.00	T ₂ 13.50	T ₃ 27.00	T ₄ 40.50	SEM
Semen volume (ml)	0.33	0.33	0.30	0.32	0.02
pH	7.00	7.10	7.03	7.03	0.29
Semen Colour	Milky-white	Milky-white	Milky-white	Milky-white	Milky-white
Sperm concentration (x 10 ⁶ /ml)	1.76 ^b	1.72 ^b	1.85 ^a	1.96 ^a	0.05
Individual motility (%)	75.03 ^c	75.17 ^c	80.03 ^b	80.23 ^a	0.08
Proportion of live sperm cells (%)	74.33 ^c	75.00 ^c	79.67 ^b	85.00 ^a	0.45
Proportion of dead sperm cells (%)	24.67 ^a	24.67 ^a	19.67 ^b	85.00 ^a	0.45

abc: Means within row having different superscript are significantly (P < 0.05) different. SEM = Standard error of means.

Turkeys on T₄ recorded the highest value of 80.23% in individual motility and this differed significantly (P < 0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₁ and T₂ were similar (P > 0.05) to each other in individual motility, but differed significantly (P < 0.05) from those on T₃. The lowest value in individual motility was observed in turkeys on T₁ (75.03%). The values for percentage of motile spermatozoa obtained in this study were higher than the range of 36-52% and 48.3-57.3% reported by Abu *et al.* (2006) and Ameh (2004) respectively in Nigerian cocks. The difference observed in sperm motility may be attributed to breed (Oguike *et al.*, 2000) and drug administration (Abu *et al.*, 2006).

Turkeys on T₄ recorded the highest percentage of live sperm cells (80.00%) and this differed significantly (P < 0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₁ and T₂ were similar (P > 0.05) to each other in percentage of live sperm cells, but they differed significantly (P < 0.05) from turkeys on T₃. The lowest percentage of live sperm cells was observed in turkeys on T₁ (75.03%). The percentages of live sperm cells obtained in this study were higher than the range of 44.2 – 59.2% reported by Abu *et al.* (2006) and higher than the range of 51.3-54.4% reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. The highest percentage of live sperm cells obtained in this study (80.00%) was higher than the average value of 79.82% reported by Oguike *et al.* (2000) in Nigerian local cocks. This disparity in the percentages of live sperm cells may be attributed to breed and drug administration (Abu *et al.*, 2006). It is suggested that high percentage of live sperm cells is vital for high fertility (Abu *et al.*, 2006). Turkeys on T₁ and T₂ recorded the highest percentage of dead sperm cells (24.67%) and these differed significantly (P < 0.05) from turkeys on T₃ and T₄ which were also significantly different (P < 0.05) from each other in percentage of dead sperm cells. The lowest percentage of dead sperm cells was observed in turkeys on T₄ (15.33%). The range of percentage dead sperm cells obtained in this study (15.33–24.67%) compares favourably with the range of 16.54 \pm 0.7–20.05 \pm 0.5% reported by Iheukwumere *et al.* (2008), and the range of 21.0–22.5% reported by Abu *et al.* (2006), but higher than the mean value of 14.25% reported by Ameh (2004) in Nigerian cocks.

The observation in this study that the group that received highest dose of the test drug recorded the highest percentage of live sperm cells, and lowest percentage of dead sperm cells suggest that a high dose of the drug such as 0.27ml/ turkey within 3 days given in this study could have high capacity for induction of spermatogenesis, improvement of semen quality and high reproductive performance in male turkeys.

The results of Dicla[®] administration on body conformation of mature male turkeys are shown in Table 4. There were significant differences (P < 0.05) among the treatment groups in all the parameters measured for body conformation; wing length, neck length, shank length, body length, beak length, thigh length, keel length, chest circumference and tail length.

Turkeys on T₄ had the highest value of 34.67cm in wing length and this differed significantly (P < 0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₂ and T₃ were similar (P > 0.05) to each other in wing length, but differed significantly (P < 0.05) from those on T₁.

Turkeys on T₄ had the highest value of 34.67cm in wing length and this differed significantly (P < 0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₂ and T₃ were similar (P > 0.05) to each other in wing length, but differed significantly (P < 0.05) from those on T₁.

Turkeys on T₄ recorded the highest value of 13.67cm in neck length and this differed significantly (P<0.05) from turkeys on T₁ and T₃ which were similar (P>0.05) to each other in neck length, but differed significantly from turkeys on T₂. There was no significant difference (P>0.05) between turkeys on T₄ and T₂ in neck length. The lowest value in neck length was observed in turkeys on T₁ (12.03cm).

Turkeys on T₃ recorded the highest value of 14.47cm in shank length and this differed significantly (P<0.05) from turkeys on T₁ which were significantly different (P<0.05) from those on T₂ and T₄ in shank on T₃, T₂ and T₄ in shank length.

Turkeys on T₄ recorded the highest value of 71.43cm in body length and this differed significantly (P<0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₂ and T₃ were similar (P>0.05) to each other in body length, but differed significantly (P<0.05) from those on T₁ which recorded the lowest value of 64.00cm in body length.

Turkeys on T₄ had the highest value of 5.97cm in beak length and this differed significantly (P<0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₂ and T₃ were similar (P>0.05) to each other in beak length. The lowest value in beak length was observed in turkeys on T₁ (5.07cm). The values of beak length obtained in this study follow a particular trend of increasing as the level of the test drug increased.

Turkeys on T₄ had the highest value of 19.03cm in thigh length and this differ significantly (P<0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₂ and T₃ were similar (P>0.05) to each other in thigh length, but differed significantly (P<0.05) from those on T₁ which recorded the lowest value of 16.17cm in thigh length.

Turkeys on T₄ had the highest value of 25.03cm in keel length and this differed significantly (P<0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₁ and T₃ were similar (P<0.05) to each other in keel length, but differed significantly (P<0.05) from those on T₂. The lowest value in keel length was observed in turkeys on T₁ (21.70cm).

Turkey on T₄ recorded the highest value of 60.06cm in chest circumference and this differed significantly (P<0.05) from turkeys on T₁, T₂ and T₃. Turkeys on T₂ and T₃ were similar (P>0.05) to each other in chest circumference, but differed significantly (P<0.05) from those on T₁ which recorded the lowest value of 55.03cm in chest circumference.

Turkeys on T₄ had the highest value of 25.10cm in tail length and this differed significantly (P<0.05) from turkeys on T₁, T₂ and T₃ which were also significantly different (P<0.05) from each other in tail length. The lowest value in tail length was observed in turkeys on T₁ (22.02cm).

The observation in this study that the group that received the highest dose of the test drug recorded the highest values in all the parameters for body conformation suggests that a high dose of Diclaire® such as 0.27ml/turkey within 3 days given in this study could have increased metabolism and efficient utilization of nutrients that resulted in improved body conformation of the turkeys.

TABLE 4
EFFECT OF DICLAIR® ON BODY CONFORMATION OF MATURE MALE TURKEYS

Parameters	Treatment (Diclaire® i.u)				
	T ₁ 0.00	T ₂ 13.50	T ₃ 27.00	T ₄ 40.50	SEM
Wing length (cm)	28.50 ^c	31.53 ^b	31.77 ^b	34.67 ^a	1.08
Neck length (cm)	12.03 ^b	13.17 ^a	12.70 ^b	13.67 ^a	0.22
Shank length (cm)	13.43 ^b	14.07 ^a	14.47 ^a	14.12 ^a	0.12
Body length (cm)	64.00 ^c	67.23 ^b	68.83 ^b	71.43 ^a	1.17
Beak length (cm)	5.07 ^b	5.20 ^b	5.12 ^b	5.97 ^a	0.21
Thigh length (cm)	16.17 ^c	17.17 ^b	17.10 ^b	19.03 ^a	0.12
Keel length (cm)	21.70 ^c	23.20 ^b	22.10 ^c	25.03 ^a	0.20
Chest circumference (cm)	55.03 ^c	57.07 ^b	55.70 ^b	60.06 ^a	0.15
Tail length (cm)	22.02 ^d	23.10 ^c	23.38 ^b	25.10 ^a	0.02

abcd: Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

The results of Diclaire® administration on hormonal profile of mature male turkeys are shown on Table 5. There were significant differences ($P < 0.05$) among the treatment groups in all the parameters measured for hormonal profile: follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone.

Turkeys on T_1 recorded the highest value of 15.09 i.u/L in follicle stimulating hormone and this differed significantly ($P < 0.05$) from turkeys on T_1 and T_2 which were similar ($P > 0.05$) to each other in FSH values. There was no significant difference ($P > 0.05$) between turkeys in T_1 and T_4 in FSH values. The lowest value in FSH was observed in turkeys on T_2 (10.76 i.u/L). The FSH values obtained in this study were higher than the range of 0.80 ± 0.08 i.u/L 1.34 ± 0.1 i.u/L reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This may be attributed to differences in breed and drug administration (Culbner *et al.*, 1977). Turkeys on T_1 recorded the highest value of 8.35 i.u/L in LH and this differed significantly ($P < 0.05$) from turkeys on T_2 , T_3 and T_4 . Turkeys on T_2 and T_3 were similar ($P > 0.05$) to each other in LH values, but differed significantly ($P < 0.05$) from those on T_4 . The lowest value in LH was observed in turkeys on T_2 (6.30 i.u/L). Luteinizing hormone (LH) as interstitial cell stimulating hormone (ICSH) stimulates the interstitial cells of Leydig to produce testosterone which facilitates the process of spermatogenesis (Herbert *et al.*, 2002). The values of FSH obtained in this study were higher than the range of 0.65 ± 0.04 – 1.36 ± 0.04 i.u/L reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This may be attributed to differences in breed and drug administration (Herbert *et al.*, 2002).

Turkeys on T_4 recorded the highest value of 0.89 (ng/ml) in testosterone and this differed significantly ($P < 0.05$) from turkeys on T_1 and T_2 which were similar ($P > 0.05$) to each other in testosterone values, but differed significantly from turkeys on T_3 . The lowest value of 0.26 ng/ml was observed in turkeys on T_1 . There was no significant difference ($P > 0.05$) between turkeys on T_4 and T_3 in testosterone values. The testosterone values obtained in this study were higher than the range of 0.15 ± 0.01 ng/ml – 0.21 ± 0.21 ng/ml reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This could be attributed to differences in breed of the birds as well as drug administration (Herbert *et al.*, 2002).

TABLE 5
EFFECT OF DICLAIR® ON HORMONAL PROFILE OF MALE TURKEYS

Parameters	Treatment (Diclaire® i.u)				
	T_1 0.00	T_2 13.50	T_3 27.00	T_4 40.50	SEM
FSH (iu/L)	15.09	10.76	11.24	7.06	0.39
LH (iu/L)	8.35	6.30	6.48	7.06	0.35
Testosterone (ng/ml)	0.26 ^b	0.34 ^b	0.64 ^{ab}	0.89 ^a	0.14

abcd: Means within row having different superscript are significantly ($P < 0.05$) different. SEM = Standard error of means.

Follicle stimulating hormone has been associated with the control of seminiferous tubule growth and differentiation (McDonald, 1975). It would seem that turkeys on T_1 and T_4 may be more efficient in sperm production since spermatogenesis takes place in the seminiferous tubules of the testis.

IV. CONCLUSION

The results of the study showed that Diclaire® improved semen quality and body conformation of the male turkeys at the level of 40.50 i.u without any deleterious effects on hormonal profile.

The levels of the hormones fall within the normal ranges for adult birds, the variations observed suggest the need to constantly monitor hormonal profile of male turkeys under Diclaire® treatment.

REFERENCES

- [1] Abu, A.H., M. Ameh, and Iheukwumere, (2006). Semen quality of Nigerian Local cock reared with human menopausal gonadotrophin (Pergonal®) Livestock Research for Rural Development.
- [2] Ameh, M. (2004). Effect of Pergonal® on semen quality, haematological values and carcass characteristics of the Nigerian Local Cocks. M.Sc. Thesis. Department of Animal Science and Fisheries Abia State University, Umuahia, Nigeria.
- [3] Brant, A.W. (1998). A brief history of turkey. World's Poultry Science 44: 365-375.
- [4] Chalov, (1970). Semen quality and fertilizing capacity of cocks ptiferodstron 20 (1): 24-26.
- [5] Culbner, J.P., T. Sharp, and J.W. Wells, (1977). Concentrations of Testosterones and LH in the blood before and after the onset of spermatogenesis in the cockerel Report. Fertile, 51:153.

- [6] Dixon, T.A. and G.J. Hopkins, (1996). Super ovulation in cattle using porcine pituitary gonadotrophin preparation (Plusset Serono) in: Plusset Scientific Literatre Serono Veterinary, Rome, Italy, pp. 22-23.
- [7] Ezekwe, A.G. I.J. Udozor, and Osita, (2003). Effect of quantitative Feed Restriction on semen quality of Nig. Local Cocks. *Nig. J. Anim. Prod.* 2003, 30 (1): 127 – 132.
- [8] Herbert, U., P. Okoro, D.O. Umesiobi, and M.U. Iloeje, (2000). Effects of two preparations of clomiphene citrate on the super-ovulation of West African Dwarf Ewes. 14th int. Congr. on Anim. Reprod. Sweeden. 2:114.
- [9] Herbert, U., A.H. Ezeobi, and Iloeje, M.U. (2002). Induction of Spermatogenesis in Rabbits using fertility drug clomiphene citrate (Clomid®), Proc. 27th Ann. Conf. NSAP, March 17 – 27.
- [10] Iheukwumere, F.C., U. Herbert, and D.O. Umesiobi, (2001). Biochemical Evaluation of Seminal Plasma in Yankasa Rams under Different Intensities of semen collection. *Int. J. Agric Rural Dev.* 2:29 – 34.
- [11] Iheukwumere, F.C., U. Herbert, and M.U. Iloeje, (2004). Haematological and Serum Biochemical Values of West African Dwarf does following FSH + LH (Pergonal®) Treatment, *Int. J. Agric. Rural Dev.*, 5:54 – 60.
- [12] Iheukwumere, F.C. (2005). Super Ovulation in Goats In: Afam Anene and Nwaigbo, L.C. (eds). *Issues in Sustainable Agriculture in Nigeria* – Osprey publication centre, Owerri, Nigeria, 1 – 9.
- [13] Iheukwumere, F.C., A.H. Abu, and E.C. Ndubuisi, (2008). Effect of FSH + LH (Pergonal®) treatment on haematology, immune status and serum metabolites of West African Dwarf Goats. *Journal of Animal and Veterinary Advances* 7(1): 46-50.
- [14] Katie, T. and A. Frazer, (1998). *The complete book of raising livestock and poultry* Macmillian Publishers Ltd.
- [15] McDonald, L.E. (1975). *Female Reproductive system in Veterinary Endocrinology and Reproduction*, Lea and Ferbiger, Philadelphia, pp. 276 – 278.
- [16] Meacham, R. (2002). Perspectives and Editorials *Andrologia* J.A. Androl. 23: 330-331.
- [17] Micallef, L.A., M.M. Hays, A. Latif, R. Alhasan, and S.B. Sufi, (1995). Serum binding of Steroid Tracers and its possible effects on Direct Steroid Immuno assay. *Ann. Cli. Biochem.* 32: 566-574.
- [18] Obi, I.U. (1990). *Statistical methods of Detecting Differences between treatment means*. Snaap press 2nd Ed. Enugu. Nigeria 24-35.
- [19] Oguike, M.A., A.N. Ndubueze, and S.N. Ibe, (2000). Semen quality of different genotypes of Nig. Local Cocks *J. Sustain. Agric. Environ.* 2(2): 310-313.
- [20] Okeudo, N.J. (2005). Empirical Studies of the living condition of domestic animals in Nigeria results from U.C. Amalu and Gottwal, F. (eds): *Studies of sustainable Agriculture and Animal Science in sub sahara Africa*. Peter Lang. Europals Cher Verlag der Wissen Shaffen, Germany.
- [21] Ozkan, S., P. Settar, and S. Yalun, (1998). Effect of Seasonal Ambient Temperature on carcass characteristics of Naked Neck and Normal feathering bird. *Animal breeding abstract*. 66-361.
- [22] Probakaran, R. (2003). Good practices in Planing and Management of Integrated commercial Poultry Production South Asia FAO Animal Production and Health Paper 159, pp. 71-86.
- [23] Rodriguez – Martinez, H. and A.D. Barth, (2007). *In vitro Evaluation of sperm Quality related to in vivo function and fertility in: Reproduction in Domestic Animals VI*. Edited by J.I. Juengel J.E. Murray and Mi Smith. Nottingham University press. Nottingham U.K. pp. 39-54 SOC Reprod. Fert. 64:39-54.
- [24] Steel, R.G.D. and J.H. Torrie, (1980). *Principles and Procedures of Statistics A. Biometric Approach* 2nd Ed. Mc. Graw-Hill Book Co. Inc. New York.